Response of Liver and Heart Trace Elements in Rats to the Interaction Between Dietary Zinc and Iron

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ABSTRACT

An analysis of the interaction between dietary iron (Fe) and zinc (Zn) was performed by using data from Sprague–Dawley rats in a 5×4 fully crossed factorially arranged experiment. The concentrations of 9 trace elements from the liver and 10 from the heart were determined and subjected to diverse statistical analyses and were classified by their response to the interaction between dietary Fe and Zn. The interaction was studied by using analysis of variance (ANOVA), discriminant analysis, and logistic regression to determine the direction of interaction; that is, did dietary Fe affect dietary Zn or did dietary Zn affect dietary Fe? The use of discriminant analysis allowed for using multiple parameters (rather than a single parameter) to determine possible interactions between Fe and Zn. Thus, two main levels of interaction were studied: the separate response of each tissue mineral and the response of some grouped minerals. The responses studied were the effect of dietary Zn on tissue trace element parameters, the effect of dietary Fe on the parameters, the effect of dietary Zn on combined (grouped) parameters, and the effect of dietary Fe on combined parameters. As determined by ANOVA, only three individual trace elements—liver Fe, Cu, and Mo—were significantly affected by the interaction between Fe and Zn. However, a broader interaction between Fe and Zn is revealed when groups of, rather than single, trace elements are studied. For example, an interaction between dietary Fe and Zn affects the weighted linear combination of heart Ca, Cu, K, Mg, Mn, P, and Zn. This article presents the hypothesis that grouped parameters may be useful as status indi-

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cators. The complete dataset can be found at http://www.gfhnrc.ars. usda.gov/fezn.

Index Entries: Interaction; zinc; iron; discriminant analysis; analysis of variance.

INTRODUCTION

Zinc (Zn) and iron (Fe) are essential for normal growth and development, and are required in milligrams-per-day quantities (e.g., 10 mg Fe/d and 15 mg Zn/p d for a male adult human) *(1).* Numerous studies have demonstrated that the capacity of Fe absorption in rodents is affected by dietary Zn and vice versa *(2).* However, Zn and Fe do not interact in humans to the same magnitude as in rodents; for example, rats do not discriminate between heme and nonheme Fe *(2).* Under normal conditions, the nature and extent of Zn complex formation with food determine Zn absorption in humans; normally, the influence of Fe on Zn absorption is not significant. However, if large doses of supplemental Fe are ingested in the absence of food, it is likely that Fe could impair Zn absorption *(2).* Solomons and Jacob showed that the evidence for competitive interaction between zinc and iron was strongest with nonheme iron and inorganic zinc at Fe/Zn ratios of 2 : 1 or 3 : 1 *(3).* Zn is known to interfere with Fe uptake by the liver; this can result in a decrease in the storage of Fe as ferritin *(2).*

Both Fe and Zn appear in the first transition series of the periodic table, and they share an identical outer electronic configuration with manganese, cobalt, and nickel. Hill and Matrone *(4)* provided a theory suggesting that elements whose physical and chemical properties are similar will interact antagonistically. These properties depend largely on the outermost electronic structure and are generally defined by the groups in the periodic table. Elements in a given group are similar in chemical nature and might be expected to compete for the same binding ligands that promote absorption or functional site. Although the similarity of electronic structure has proven to be a useful guide, it does not explain all of the observed nutritional interactions.

We describe the interaction between dietary Fe and dietary Zn in rats with a focus on trace element data from the liver and heart. Our objective was to study the qualitative nature of the interaction. To be statistically representative, the experiment utilized a sufficiently large number of animals (*N*=119). We implemented a combination of diverse statistical tools to facilitate the analyses of the interaction. We further studied the interaction by determining the direction of the interaction (whether Zn affected Fe or whether Fe affected Zn).

Table 1
Composition of the Basal Diet[a]

Ingredient	g/kg
Cornstarch	399.5
Spray-dried egg white	200.0
Dextrinized cornstarch	132.0
Sucrose	100.0
Soybean oil	70.0
Cellulose	50.0
Mineral mix[b]	35.0
Vitamin mix	10.0
Biotin mix	1.0
Choline bitartrate	2.5
Total:	1000.0

[a] Diet formulation based on AIN-93 Growth—Egg White *(5)*; diet contains no tert-butylhydroquinone.
[b] Basal diet contains no added Fe, or Zn; it provides Cu at 4 µg/g diet.

MATERIALS* AND METHODS

Male weanling Sprague–Dawley rats (Sasco, Inc.) were weighed individually upon arrival. Six rats were assigned to each dietary group ($N=5$ for 96 µg Fe/g and 20 µg Zn/g group) with no significant difference in initial weight among groups. The rats were housed in hanging stainless-steel cages. Room temperature was maintained at 23°C and humidity at 50%. Automatically controlled lighting provided 12 h light daily. Animals were weighed and provided clean cages weekly. The study was a 5 × 4 fully crossed factorially arranged experiment. Dietary variables were Fe (as ferric citrate) at 4, 12, 24, 48, or 96 µg/g diet and Zn (as Zn carbonate) at 5, 10,

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20, or 40 µg/g diet. The diet (Table 1) was based on the AIN-93 formulation for an egg white protein source *(5)*. Feeding diets low in Zn can cause cyclical feeding *(6)*. The lowest concentration of Zn (5 µg/g) fed in this experiment did not cause cyclical feeding, as indicated by food consumption (data not shown). Feed efficiency was similar throughout the experiment for all dietary groups (data not shown). Thus, any effect of dietary treatment on parameters studied was not the result of differential food intake. Animals had free access to food and deionized water (Super Q Systems, Millipore Corp., Bedford, MA). After 7 wk, tissues were collected and weighed. The concentrations of Ca, Cu, Fe, K, Mg, Mn, Mo, P, and Zn were measured in the liver and heart; Na was also measured in the heart *(7, 8)*.

Data were statistically processed by using analysis of variance (ANOVA), discriminant analysis, and logistic regression found in the statistical package SAS *(9)*. The different statistical methods were compared and confidence levels were calculated. The complete dataset can be found at http://www.gfhnrc.ars.usda.gov/fezn.

RESULTS

Interaction Analysis

Traditionally, the interaction source of variation within a population can be evaluated by ANOVA; these results are presented in Table 2 (liver trace elements) and Table 3 (heart trace elements).

These data (Table 2) show that an interaction between dietary Fe and Zn affects liver Cu, Fe, and Mo. For the heart data, the hypothesis must be rejected with very high probability for all measured trace elements (Table 3). There might be several reasons for finding only limited effects of an interaction in liver and no significant interaction in heart; these include that the interaction is very specific, the mathematical tool used was improper, or the variances were nonhomogeneous.

A different mathematical approach using combined heart trace element data shows, with a very high probability, an interaction between Fe and Zn (see below). Thus, the interaction will be reflected in a group of biochemical parameters, rather than in a single one. This group can be interpreted later as a factor of interaction [see traditional factor analysis *(10)*]. To create a proper combination of parameters, the heart trace element data were subjected to discriminant analysis *(11,12)* using dietary Zn as a classification variable. Discriminant analysis is able to provide a linear combination of original parameters with the highest response to the classification variable (dietary intake of Zn, in this case). The purpose of discriminant analysis is to define, for a set of parameters, a small number of linear combinations that summarize between class (e.g., between dietary groups) variation and retain as much of the information of the original parameters as possible. The coefficients of the linear combination of original data are

Table 2
ANOVA on the Interaction Between Dietary Fe and Zn on the Concentration
of Trace Elements in Liver

Trace Element	Ca	Cu	Fe	Mg	Mn	Mo	P	Zn	K
Fe x Zn, p value[a]	0.19	0.0001	0.0001	0.74	0.10	0.019	0.69	0.48	0.74

[a] Significance measured at $p<0.05$.

Table 3
ANOVA on the Interaction Between Dietary Fe and Zn on the Concentration
of Trace Elements in Heart

Trace Element	Ca	Cu	Fe	Mg	Mn	Mo	P	Zn	K	Na
Fe x Zn, p value[a]	0.70	0.86	0.84	0.54	0.68	0.81	0.55	0.23	0.98	0.94

[a] Significance measured at $p<0.05$.

canonical coefficients or canonical weights and are weighted to maximize the difference among the treatments (dietary groups). The variable defined by the linear combination is a canonical component (CAN). The first canonical variable (CAN1) provides the maximal multiple correlation. The second canonical variable (CAN2) provides the next most maximal multiple correlation. The weighted linear combination of biochemical parameters was calculated as follows:

$$CAN1 = X1*Ca+X2*Cu+X3*Fe+X4*Mg+X5*Mn+X6*Mo+X7*P+X8*Zn+X9*K+X10*Na$$

where Xi is the canonical weight coefficient for each measured trace element and each trace element symbol represents the tissue concentration of the trace element. The canonical weighting coefficients for the first canonical variable (CAN1) are presented in Table 4.

The ANOVA evaluation of the interaction between dietary Fe and Zn for CAN1 was $p<0.019$. Although this method shows that an interaction between Fe and Zn affects heart trace element content, little light is shed on the mechanism of the interaction itself.

In the following, a consistent method is presented to evaluate the interaction between dietary components. The results show that the effect of the dietary interaction was present in both liver and heart and its range was very broad within the 90% confidence level.

After showing that an interaction between Fe and Zn existed, the next step was to determine whether dietary Zn was able to affect Fe (denoted by Zn → Fe) with dietary Fe having a minor or no affect on Zn, or vice versa (denoted by Fe → Zn), or if each dietary variable was able to affect the response of the counterpart (Zn ↔ Fe). The results, as obtained by

Table 4
Canonical Weighting Coefficients for the Heart Trace Element Data

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
CAN1	-0.03	0.31	0.01	-0.01	3.97	1.16	0.00	-0.22	0.00	0.00

using data from trace element analysis of liver and heart, show that dietary Zn affects Fe and also that dietary Fe affects Zn. Thus, the interaction between these two elements, as expressed in the concentration of trace elements in liver and heart, can be denoted by Zn ↔ Fe. Following is the method used in these analyses.

The more an organ (i.e., the trace element concentration in liver or heart) is responsive to a dietary component (Fe or Zn), the better the ability to identify a priori unknown dietary intake of the component based on the concentration of trace elements in the tissue. Therefore, the strength of the connection between dietary components and the trace element concentration data was measured by the probability to predict the dietary concentration of Fe or Zn by using only the trace element data (the concentration of 9 trace elements from the liver and 10 from the heart). We investigated all possible combinations of trace elements (Ca, Cu, Fe, K, Mg, Mn, Mo, P, and Zn for liver, $2^9 - 2 = 510$ combinations total; and Ca, Cu, Fe, K, Mg, Mn, Mo, P, Zn, and Na for heart, $2^{10} - 2 = 1022$ combinations total). Logistic regression *(9,13)* was used to calculate the probability function forecasting dietary Fe or dietary Zn concentration based on these combinations. The ability to determine the dietary components was assessed by a percent of successful prediction, which was calculated by a logistic procedure. This procedure simulated a blinded computer experiment where the dietary groups were assumed unknown but the trace element data were available. The value of the successful prediction is called the concordant value or concordant *(9,13)*.

Statistical Analysis of Liver Data

First, the ability to predict the dietary Fe concentration based on the liver data was determined; this was divided into four substeps. The results are presented in Tables 5–7.

> **Step 1.** Table 5 presents all combinations of liver trace elements excluding Fe that resulted in a greater than 81% ability to predict dietary Fe. We observed that the single trace element (not including Fe) that was best able to predict dietary Fe was Mo. This element alone had a concordant value of 81.2% and was present in all other successful (>81%) combinations as listed in Table 5.
>
> **Step 2.** Next, all possible combinations of trace elements (including Fe) that resulted in concordant values greater than 92.5% were

Table 5
Best Predictors (81% and More) of Dietary
Fe from Liver Trace Element Data
Not Including Liver Fe

Concordant %	Ca	Cu	Mg	Mn	Mo	P	Zn	K
82.9	1	0	0	1	1	1	0	1
82.8	0	0	0	1	1	1	0	1
82.8	0	0	1	1	1	1	0	0
82.4	0	0	0	0	1	0	0	1
82.4	0	0	0	1	1	0	0	1
82.3	1	0	1	1	1	1	0	1
81.2	0	0	0	0	1	0	0	0
81.2	1	0	1	1	1	1	1	1

In the table, a "1" means that the trace element in that particular row is included in deriving the concordant %; a "0" means that the trace element in that particular row is not included in deriving the concordant %. For example, in the first row, the combination of Ca, Mn, Mo, P, and K was used to derive an 82.9% prediction of dietary Fe.

determined (Table 6). The combination of liver Fe and P brought the highest value of 94.5%. The concentration of liver Fe alone was able to predict dietary Fe with concordant value of 91.9%.

Step 3. The population of 119 rats was divided according to dietary Zn. For each of these four subpopulations (5, 10, 20, and 40 µg Zn/g), the percent of successful dietary Fe prediction based on liver Fe only, liver Fe and P pair (see Step 2), and all other combinations of trace elements listed in Table 5 were calculated. It was reasoned that some concentrations of dietary Zn might affect the liver response (trace element concentration) to dietary Fe differently than other concentrations of dietary Zn, signifying an interactive effect. In other words, this is used to determine if dietary Zn was able to affect Fe (denoted by Zn → Fe). The corresponding data are presented in columns 3–6 of Table 7. Naturally, because the animals are subdivided by dietary Fe, there are fewer animals used as opposed to the predictions outlined in

Table 6
Best Predictors (Greater than 92.5%) of
Dietary Fe from Liver Trace Element Data
Including Liver Fe

Concordant	Liver trace element								
%	Ca	Cu	Fe	Mg	Mn	Mo	P	Zn	K
94.5	0	0	1	0	0	0	1	0	0
93.9	0	0	1	1	0	0	1	0	0
93.8	0	0	1	1	1	0	1	0	0
93.5	0	0	1	0	1	0	1	0	0
92.8	0	0	1	1	1	0	1	0	1
92.8	1	0	1	0	1	0	0	1	0
92.7	0	0	1	1	0	0	1	0	1
92.7	0	0	1	0	1	0	0	1	0
92.7	1	0	1	0	0	0	1	0	0
92.6	1	0	1	1	1	0	1	0	0
92.6	1	0	1	0	1	0	0	0	0

Note: See Table 5 for explanation of "0" and "1".

Steps 1 and 2; therefore, the concordant values are less for these predictions. Visually, it can be seen that there are substantial differences in the concordant values when the concordant is determined over the dietary range of Zn concentrations.

Step 4. The 90% and 95% confidence intervals of the concordant values (percent successful dietary predictions as determined in Step 3 and shown in columns 3–6) for all combinations of trace elements listed under the column entitled "parameter" (column 1, Table 7) were calculated by using the *t*-distribution. This was done to determine the significance of the variations in the percent successful dietary predictions of dietary Fe as classified by dietary Zn. The results are presented in columns 7–10 ("Confidence Interval") of Table 7. Comparing the numbers in columns 3–6 with the lower and upper boundaries from columns 7–10, it can be seen that no single combination was outside the 95% confidence interval. The combinations Fe,

Table 7

Statistical Analysis of Liver Parameters by Using Logistic Regression to Evaluate the Effects of Dietary Zn on Dietary Fe

Parameter	mean[a]	Dietary Zn concentration, µg/g				Confidence Interval				P value from ANOVA			
		concordant, %				90%		95%		Fe		Zn	
		5	10	20	40	lower	upper	lower	upper	CAN1[b]	CAN2[b]	CAN1[b]	CAN2[b]
Fe	75.10	90.00	71.40	69.30	69.70	63.36	86.84	59.23	90.97	0.0001	0.0001	0.0001	0.0001
Fe+P	75.65	86.90	75.00	72.90	67.80	66.13	85.17	62.78	88.52	0.0001	0.575	0.0001	0.661
Mo	76.30	65.00	80.70	79.50	80.00	67.42	85.18	64.29	88.31	0.0001	0.0001	0.0001	0.0001
Mo+K	73.55	53.00	82.70	80.40	78.10	57.28	89.82	51.55	95.55	0.03	0.345	0.54	0.007
Mn+Mo+K	72.53	53.00	81.80	79.50	75.80	56.93	88.12	51.44	93.61	0.008	0.043	0.791	0.029
Mg+Mn+Mo+P	77.70	72.80	85.10	72.30	80.60	70.37	85.03	67.79	87.61	0.01	0.369	0.058	0.723
Mn+Mo+P+K	72.33	58.50	81.30	66.40	83.10	58.35	86.30	53.43	91.22	0.0157	0.261	0.77	0.028
Ca+Mn+Mo+P+K	73.95	63.90	81.30	66.40	84.20	61.85	86.05	57.59	90.31	0.028	0.0455	0.939	0.101
Ca+Mg+Mn+Mo+P+K	74.15	63.90	82.40	66.40	83.90	61.84	86.46	57.51	90.79	0.031	0.19	0.952	0.08
Ca+Mg+Mn+Mo+P+Zn+K	74.95	63.90	83.90	66.4	85.60	61.56	88.34	56.84	93.06	0.126	0.233	0.425	0.073

[a] Mean of concordant values over all dietary zinc concentrations of parameter listed in column 1.
[b] CAN (canonical variable); CAN1 and CAN2 are linear combinations of parameters (listed in column 1) based on discriminate analysis.
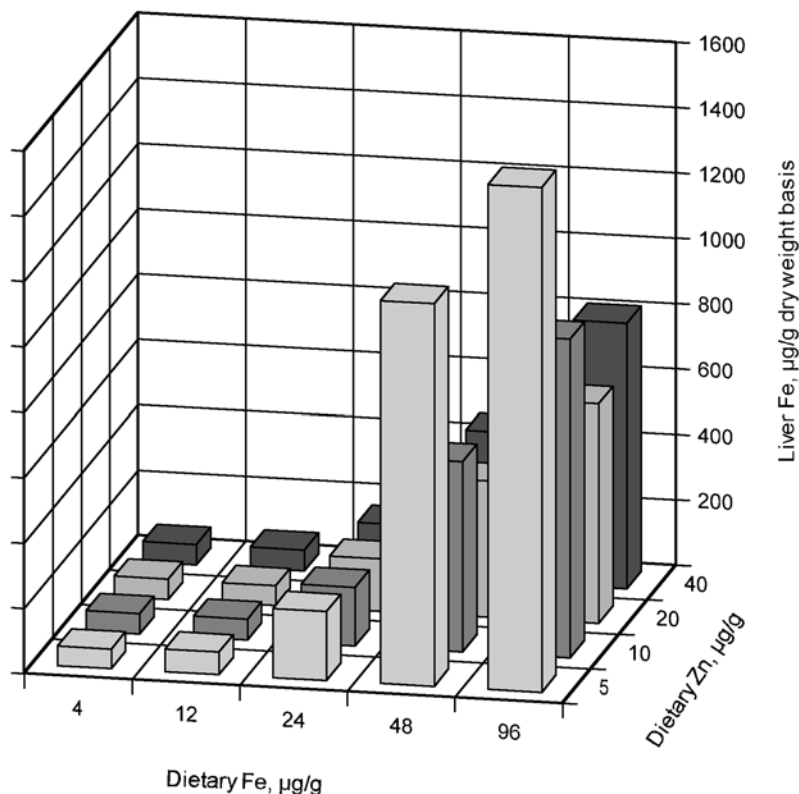
Fig. 1. Concentration of Fe in liver of rats fed diets containing graded amounts of Fe and Zn.

Fe+P, Mo, Mo+K, Mn+Mo+K, and Mg+Mn+Mo+P, however, exceeded the 90% confidence interval. Therefore, it was concluded that the data confirmed the interaction Zn → Fe with 90% confidence. This interaction was reflected in various combinations of liver Fe, P, Mn, Mo, and K. The ANOVA portion of Table 7 (columns 11–14) is explained later.

There are additional considerations needed when discussing the response of liver Fe to the interaction between dietary Fe and Zn. In spite of the high probability of the interaction between Fe and Zn shown by ANOVA (Table 2), the logistic regression procedure to determine interactions was only shown for the 90% confidence interval (see Table 7). The main reason for this is the large range of values (60–1545 µg/g) for liver Fe (Fig. 1). The numerical algorithm of the logistic procedure was unable to properly fit this irregular data. Therefore, the conditional probability of dietary Fe for a given liver concentration of Fe and dietary Zn was calculated by using the Genmod procedure *(9)*. The Genmod procedure is able to calculate the conditional probability of categorical events (dietary

groups) for the given numerical data (values of liver Fe) and categorical data (dietary Zn). Logistic regression can only use numerical explanatory variables such as liver Fe. Unfortunately, the current Genmod* procedure cannot model more than two events. Because of this, all Fe diets were separated into two groups: group 0 (dietary Fe=4, 12, 24, and 48 µg/g) and group 1 (Fe=96 µg/g). The generalized linear regression between dietary Fe groups (0 or 1) and the explanatory variables such as liver Fe was calculated. Results showed that the model fit the experimental data very well (deviance/degree of freedom [DF]=0.34 and chi square=59 with DF=113). Type 3 statistics (ANOVA) evaluated the zero hypothesis for regression coefficients on liver Fe and dietary Zn. The probability of no influence of liver Fe was less than 0.0001 and probability of no influence of dietary Zn was less than 0.0001. Therefore, the zero hypothesis can be rejected. Thus, it was concluded that the interaction Zn → Fe was shown for liver Fe with high probability ($p<0.001$).

Next, the ability to predict dietary Zn based on the liver data was determined. The best predictions of dietary Zn by the liver trace element data are presented in Table 8. No strong connection between the dietary Zn and the liver trace elements were found (the highest level of successful prediction is 74.6% and the flipping-coin benchmark is 50%). Therefore, based on a successful prediction cutoff of approx 80%, it was concluded that the liver trace element data would bring no reliable information about the influence of Fe on Zn (Fe → Zn). The preceding used the average population data disregarding the stratification by dietary Fe. As with liver Fe, the elements Mo and Cu that were influenced by the interaction between Fe and Zn (see ANOVA data, Table 1) cannot be characterized properly by the average data because of the very large range of means (see Figs. 2 and 3). Motivated by the ANOVA data, we calculated the ability of these elements to predict dietary Zn by using the data as grouped by dietary Fe. The results are presented in Table 9 and it can be seen that prediction of dietary Zn based on either liver Mo or Cu was influenced by dietary Fe (Fe → Zn) with 95% confidence.

Statistical Analysis of Heart Data

The trace element data from the heart was subjected to similar analyses. The results showed that the heart trace elements were able to predict dietary Fe with success 80% and above in 170 combinations (all data not shown; see Table 10 for 84% and above). It is interesting to note that the best predictors of dietary Fe, which hit 84% and above, did not use the heart Fe data but, instead, included Ca, Cu, Mn, P, and K, which were common in all of the combinations.

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*SAS 8.0 and higher provides the logistic regression that can handle numerical and categorical explanatory data as well as more than two categorical states in the independent variable (in our case, five dietary Fe groups). These opportunities were not available when our paper was written.

Table 8
Best Predictors (74% and More) of Dietary Zn
from Liver Trace Element Data

Concordant	Liver trace element								
%	Ca	Cu	Fe	Mg	Mn	Mo	P	Zn	K
74.6	1	1	0	1	1	1	1	1	1
74.5	1	1	1	1	1	1	1	1	1
74.5	1	1	1	1	0	1	1	1	1
74.5	1	1	0	0	1	1	1	1	1
74.3	1	1	0	1	1	0	1	1	1
74.2	1	1	0	0	1	0	1	1	1
74.2	1	1	1	0	1	1	1	1	1
74.2	1	1	1	0	0	1	1	1	1
74.1	1	1	0	0	0	1	1	1	1
74.0	1	1	0	0	0	0	1	1	1
74.0	1	1	1	0	0	0	1	1	1

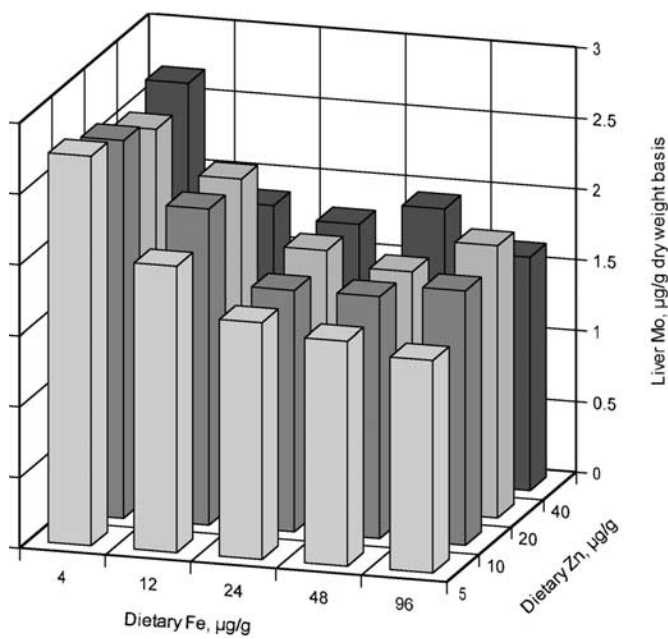*Note:* See Table 5 for explanation of "0" and "1".

Fig. 2. Concentration of Mo in liver of rats fed diets containing graded amounts of Fe and Zn.
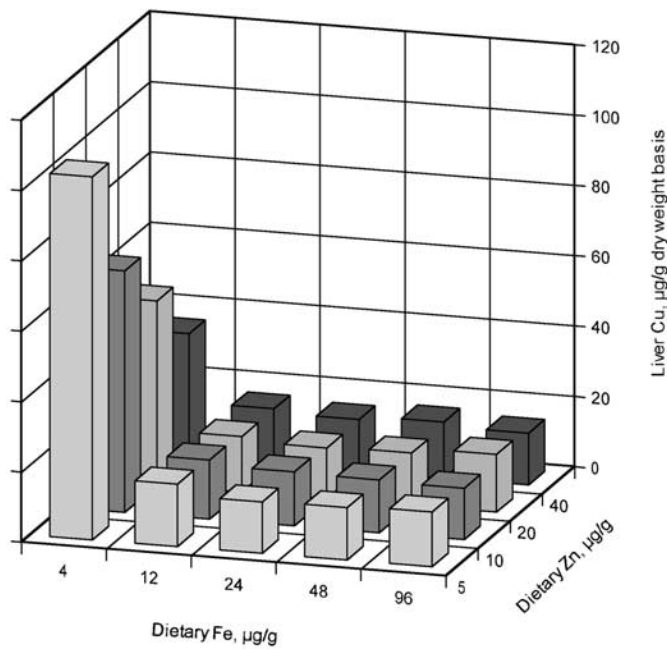
Fig. 3. Concentration of Cu in liver of rats fed diets containing graded amounts of Fe and Zn.

Table 9
Statistical Analysis of Liver Parameters by Using Logistic Regression
to Evaluate the Effects of Dietary Fe on Dietary Zn

		Dietary Fe concentration, μg/g					Confidence Interval				
		4	12	24	48	96	90%		95%		ANOVA
Parameter	mean[a]			concordant, %			lower	upper	lower	upper	p value
Mo	69.56	50.0	65.0	88.4	72.2	72.7	56.3	82.8	52.3	86.8	0.0179
Cu	78.28	74.1	72.2	87.9	64.4	77.8	67.1	77.8	64.6	85.9	0.0001

[a] Mean of concordant values over all dietary iron concentrations of parameter listed in column 1.

The top 10 combinations, none of which included Fe, that brought the population average of 84% and above were used to investigate the influence of Zn on Fe. In other words, the combinations were used to determine if dietary Zn was able to affect Fe (denoted by Zn → Fe). As before, all rats were divided according to dietary Zn (5, 10, 20, and 40 μg/g). For each group, the percent of successful Fe prediction based on the top 10 combinations of heart trace element data was calculated. The results are

Table 10
Best Predictors (84% and More) of Dietary Fe from
Heart Trace Element Data

Concordant	Heart trace element									
%	Ca	Cu	Fe	Mg	Mn	Mo	P	Zn	K	Na
84.4	1	1	0	1	1	0	1	1	1	0
84.3	1	1	0	1	1	0	1	0	1	1
84.3	1	1	0	1	1	1	1	0	1	1
84.2	1	1	0	0	1	1	1	0	1	1
84.2	1	1	0	0	1	0	1	0	1	1
84.1	1	1	0	1	1	1	1	0	1	0
84.1	1	1	0	1	1	0	1	1	1	1
84.0	1	1	0	1	1	1	1	1	1	0
84.0	1	1	0	0	1	1	1	1	1	0
84.0	1	1	0	1	1	1	1	1	1	1

Note: See Table 5 for explanation of "0" and "1".

presented in Table 11, columns 3–6. Comparing the numbers in columns 3–6 with the lower and upper boundaries from columns 7–10 ("Confidence Interval"), we can see that no single combination was outside of the 95% confidence interval. The 90% confidence interval was exceeded by combinations Ca+Cu+Mg+Mn+P+Zn+K, Ca+Cu+Mg+Mn+Mo+P+K+Na, Ca+Cu+Mn+P+K+Na, Ca+Cu+Mn+Mo+P+Zn+K, Ca+Cu+Mg+Mn+Mo +P+K, Ca+Cu+Mg+Mn+Mo+P+Zn+K+Na, and Ca+Cu+Mg+Mn+P+Zn +K+Na. Therefore, it was concluded that the heart trace element data confirmed the interaction (Zn → Fe) with 90% confidence.

The top 11 predictors of dietary Zn by the heart trace element data for the Fe → Zn interaction are presented in Table 12. As found with the liver data and by using a success of prediction cutoff of 80%, no substantial interaction was observed and, therefore, no Fe → Zn interaction was found (concordant values of only 70.4% and less were found). Thus, the results of the heart data do not support a strong Fe → Zn interaction.

The weakness of the implemented method (for both liver and heart data) relates to the small number of Zn diet groups. As a result, the confidence intervals in Tables 7 and 11 (see columns 7–10) were calculated

Table 11
Statistical Analysis of Heart Parameters by Using Logistic Regression to Evaluate the Effects of Dietary Zn on Dietary Fe

Parameter	mean[a]	Dietary Zn concentration, µg/g concordant, %				Confidence Interval 90%		95%		P value from ANOVA Fe		Zn	
		5	10	20	40	lower	upper	lower	upper	CAN1[b]	CAN2[b]	CAN1[b]	CAN2[b]
Ca+Cu+Mg+Mn+P+Zn+K	83.20	79.40	83.00	85.10	85.30	79.98	86.42	78.84	87.56	0.380	0.720	0.016	0.310
Ca+Cu+Mg+Mn+Mo+P+K+Na	78.10	61.40	84.20	81.80	85.00	64.90	91.30	60.25	95.95	0.036	0.035	0.750	0.390
Ca+Cu+Mg+Mn+P+K+Na	85.78	86.90	83.90	84.50	87.80	83.57	87.98	82.80	88.75	0.017	0.033	0.490	0.380
Ca+Cu+Mn+Mo+P+K+Na	85.13	83.90	82.10	87.80	86.70	82.07	88.18	80.99	89.26	0.016	0.008	0.735	0.370
Ca+Cu+Mn+P+K+Na	86.35	87.20	83.60	86.30	88.30	83.99	88.71	83.16	89.54	0.004	0.046	0.850	0.370
Ca+Cu+Mg+Mn+Mo+P+K	82.63	77.50	84.20	83.00	85.80	78.38	86.87	76.89	88.36	0.017	0.033	0.430	0.320
Ca+Cu+Mg+Mn+P+Zn+K+Na	83.03	77.80	79.50	85.10	89.70	76.63	89.42	74.38	91.67	0.381	0.120	0.048	0.370
Ca+Cu+Mg+Mn+Mo+P+Zn+K	80.23	77.50	82.10	77.10	84.20	76.12	84.33	74.67	85.78	0.110	0.241	0.015	0.330
Ca+Cu+Mn+Mo+P+Zn+K	82.55	80.60	82.70	83.60	83.30	80.96	84.14	80.40	84.70	0.106	0.009	0.012	0.330
Ca+Cu+Mg+Mn+Mo+P+Zn+K+Na	76.88	61.70	81.50	77.10	87.20	64.02	89.73	59.48	94.27	0.110	0.241	0.024	0.370

[a] Mean of concordant values over all dietary zinc concentrations of parameter listed in column 1.
[b] CAN (canonical variable); CAN1 and CAN2 are linear combinations of parameters (listed in column 1) based on discriminate analysis.

Table 12
Best Predictors (70% and More) of Dietary Zn
from Heart Trace Element Data

Concordant	Heart trace element								
%	Ca	Cu	Fe	Mg	Mn	Mo	P	Zn	K
70.4	1	1	0	1	1	1	1	1	1
70.4	1	1	0	1	1	1	1	1	1
70.3	1	1	0	1	1	1	1	1	0
70.3	1	1	0	1	1	1	0	1	0
70.3	1	1	0	1	1	1	0	1	0
70.3	1	1	0	1	1	1	1	1	0
70.3	1	1	0	1	1	1	0	1	0
70.3	1	1	0	1	1	1	1	1	0
70.3	1	1	0	1	1	1	0	1	0
70.3	1	1	0	1	1	1	1	1	0
70.3	1	1	0	1	1	1	1	1	0

Note: See Table 5 for explanation of "0" and "1".

based on four numbers for each combination (see columns 3–6) and, therefore, were of low statistical reliability. Because of this, it was desirable to present additional independent statistical analyses to validate the results.

Analysis of variance was applied to a linear combination of the biochemical parameters making up each particular group as listed under "Parameters" in column 1 in Tables 7 and 11. Thus, the following formula was used (the coefficients for trace elements not used were nullified [i.e., when $X_i = 0$]); discriminant analysis *(11,12)* was used to calculate the remaining nonzero weighted coefficients.

$$CAN1 = X1*Ca+X2*Cu+X3*Fe+X4*Mg+X5*Mn+X6*Mo+X7*P+X8*Zn+X9*K+X10*Na$$

Note that Na was not measured in the liver.

Discriminant analysis with dietary Zn (or Fe) as a classification variable best reveals the difference among Zn (or Fe) dietary groups; both Zn and Fe were used as classification variables to derive canonical variables. The above formula was applied to calculate the first and second canonical variables

(discriminant functions): CAN1 and CAN2. An important property of canonical variables is that they are uncorrelated. Thus, for each row in column 1, two canonical variables for dietary Zn were calculated (CAN1, CAN2) and two canonical variables for dietary Fe were calculated (CAN1, CAN2) (Tables 7 and 11). ANOVA was used to calculate the probability of interaction between dietary Fe and Zn for all combinations [(2+2)×10=40 total combinations]. The results are presented in columns 11–14 of Tables 7 and 11. By using logistic regression, all combinations of biochemical (tissue trace element) parameters but one (the last combination in Table 7) show the interaction with 95% (by ANOVA). Logistic regression is more selective than the use of ANOVA alone and, what is more important, we were able to identify the direction of interaction.

DISCUSSION

As determined by ANOVA, an interaction between dietary Fe and Zn affected liver Fe, Cu, and Mo. Further analysis demonstrated that alternative statistical methods could give useful interpretations of experimental data. Therefore, two goals were targeted. First, the trace elements (single or grouped) with sufficiently strong cooperative response to the interaction between Fe and Zn were sought. Second, the direction of the interaction was studied; that is, it was determined whether dietary Zn was affecting Fe (Zn → Fe) while dietary Fe was not affecting Zn, or vice versa (Fe → Zn), or whether the effect was mutual (Zn ↔ Fe). All possible combinations of trace elements were studied (Ca, Cu, Fe, Mg, Mn, Mo, P, Zn, and K for liver, 2^9 –2= 510 combinations total; Ca, Cu, Fe, Mg, Mn, Mo, P, Zn, K, and Na for heart, 2^{10} –2=1022 combinations total). The response to dietary treatment was assessed by a probability to predict the dietary concentration of Fe or Zn based on the trace element content of liver or heart. The probability was calculated by a logistic procedure, which simulated a computer blind experiment where the dietary groups were assumed unknown but the tissue trace element data were available. This probability was calculated for total population (119 rats) and for subpopulations with different dietary Zn (approx 30 rats per group). The effect of the interaction Zn → Fe was assessed by the changes in the probability to predict dietary Fe when data were stratified by dietary Zn. It was found that 283 different combinations of liver trace elements were able to predict dietary Fe in 80% of the cases and 81 combinations resulted in 90% of success. The highest predictions were obtained by using the combination of Fe and P (94.5%). The most responsive liver trace elements to dietary Fe were Fe (91.9%) and Mo (81.2%).

This research has shown by using a 90% confidence interval that liver Fe or Mo alone, liver Fe + P, liver Mo+K, liver Mo+K+Mn, and liver Mg+Mn+Mo+P were affected by the interaction of dietary Fe and Zn

(Zn → Fe). Discriminant analysis was used to create linear combinations of trace elements that reflected the interaction between Fe and Zn. ANOVA was applied to these linear combinations, and in all of the above-mentioned cases (and in many others), ANOVA confirmed with greater than 0.95 probability that this interaction occurred.

The same procedure was applied to heart trace elements. It was found that 170 different combinations of trace elements predicted dietary Fe with 80% success and 10 combinations resulted in successful prediction of 84% or more. The interaction (Zn → Fe) was shown (by using a 90% confidence interval) for the following combination of trace elements. Ca+Cu+Mg+Mn+P+Zn+K, Ca+Cu+Mg+Mn+Mo+P+K+Na, Ca+Cu+Mn+P+K+Na, Ca+Cu+Mg+Mn+Mo+P+K, Ca+Cu+Mg+Mn+P+Zn+K+Na, Ca+Cu+Mn+Mo+P+Zn+K, and Ca+Cu+Mg+Mn+Mo+P+Zn+K+Na. Discriminant analysis followed by ANOVA showed that these combinations and many others resulted in a probability of interaction (Zn → Fe) of 0.95 and above.

This research explained a method where mathematically combined parameters were used to increase the success of prediction of dietary groups, based on those combined parameters. This is analogous to using more than one parameter (i.e., a number of parameters grouped together mathematically) to assess status. Typically, single parameters are used as status indicators (e.g., hematocrit or hemoglobin for iron status). An offshoot of this research is that the methods outlined in this article could be used for combining parameters to theoretically derive a better indicator of status assessment.

This experiment provided much statistically reliable information supporting an interaction between dietary Zn and Fe in which dietary Zn affected Fe (Zn → Fe) as determined by the concentration of trace elements in liver and heart. However, the experiment provided limited information supporting the Fe → Zn interaction. Also, the results also suggest that the presented approaches to studying interactive effects also may be useful for determining status indicators.

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REFERENCES

1. National Research Council, *Recommended Dietary Allowances,* 10th ed., National Academy Press, Washington, DC (1989).

2. C. T. Walsh, H. H. Sandstead, A. S. Prasad, P. M. Newberne, and P. J. Fraker, Zinc: health effects and research priorities for the 1990s, *Environ. Health Perspect.* **102 (Suppl. 2),** 5–46 (1994).

3. N. W. Solomons and R. A. Jacob, Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc, *Am. J. Clin. Nutr.* **34,** 475–482 (1981).

4. C. H. Hill and G. Matrone, Chemical parameters in the study of in vivo and in vitro interactions of transition elements, *Fed. Proc.* **29,** 1474–1488 (1970).

5. P. G. Reeves, AIN-93 purified diets for the study of trace element metabolism in rodents, in: *Methods in Nutrition Research,* R. R. Watson and I. Wolinsky, eds., CRC Press, Boca Raton, FL, pp. 3–37 (1997).

6. G. J. Fosmire, M. A. Fosmire, and H. H. Sandstead, Zinc deficiency in the weanling rat: effects on liver compostiion and polysomal profiles, *J. Nutr.* **106,** 1152–1158 (1976).

7. E. O. Uthus and R. Poellot, Effect of methionine deficiency on arsenic deprivation in rats: growth, blood parameters, organ weight/body weight ratios, and tissue trace element concentrations, *J. Trace Elements Exp. Med.* **5,** 153–164 (1992).

8. F. H. Nielsen, T. J. Zimmerman, T. R. Shuler, B. Brossart, and E. O. Uthus, Evidence for a cooperative metabolic relationship between nickel and vitamin B_{12} in rats, *J. Trace Elements Exp. Med.* **2,** 21–29 (1989).

9. SAS Institute, *SAS/STAT User's Guide, Version 6,* 4th ed., SAS Institute, Inc., Cary, NC, Vol 1–2 (1990).

10. D. F. Morrison, *Multivariate Statistical Methods,* McGraw-Hill, New York (1967).

11. D. J. Hand, *Discriminant and Classification,* Wiley, New York (1981).

12. T. W. Anderson, *An introduction to Multivariate Statistical Analysis,* 2nd ed., J Wiley, New York (1984).

13. P. D. Allison, *Logistic Regression Using the SAS System,* SAS Institute, Inc., Cary, NC (1999).